THE CONTROL OF RETINAL PIGMENT MIGRATION IN LEANDER SERRATUS

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Introduction

The movements of pigments in the eyes of crustaceans are known to be related to the illumination of the eye and to the shade of the background on which the animals are placed, but the means by which these pigment movements are controlled have been disputed for more than fifty years. Conflicting evidence has pointed on the one hand to a hormonal control of these pigment movements and on the other hand to a direct response of the pigment cells to the presence or the absence of illumination. There is also the possibility that the pigment movements are under direct nervous control, but as yet no nervous connections to the distal or the reflecting pigment cells have been traced, and the proximal retinular cells containing pigment are innervated by sensory fibres only (Parker, 1891). Recent evidence has indicated that the retinal pigment cells respond to the injection of hormones, but this does not exclude the possibility that they may also respond directly to illumination, as some of the earlier evidence indicated. The present paper is an attempt to evaluate the relative importance of hormonal control and a direct response to illumination in movements of the retinal pigments in the prawn *Leander serratus*.

In 1897 Parker described experiments on *Palaemonetes vulgaris*, in which one eye was covered and the other was exposed to direct illumination. In one experiment, one eye was covered by a mixture of Canada balsam and lampblack; another experiment consisted in placing an animal in a light-proof box in such a way that one eye projected through a hole in the side of the box and was exposed to illumination. Parker reported that, in his experiments, "the eyes exposed to the light always presented the condition normal for light, and those kept in the dark always showed an approach, more or less complete, to the condition characteristic for the dark."

Experiments comparable to those of Parker were carried out by Castle (1927), who used plaster of Paris to cover the eyes of Palaemonetes and studied the effects on the movements of the proximal pigment only. Covering of one eye or of both eyes caused complete or incomplete darkness-adaption of the covered eye, although when one eye was covered and the other was exposed, the latter always showed the condition characteristic of light-adaption.

Further experiments were carried out by Bennitt (1932) on *Palaemonetes vulgaris* and *P. exilipes*, using Castle's method of covering the eye. One third of the covered eyes showed a partial adaption to darkness, but only one in forty-five of the exposed eyes of the same animals showed an intermediate state—the rest were light-adapted.

Bennitt then explored the possibility that light might be entering the covered eye through the transparent eye-stalks. To avoid this difficulty he used opaque animals, namely Cambarus, Cancer, Carcinides, and Libinia. The results, however,

were inconclusive; in some cases the illuminated eye was light-adapted and the covered eye was partially dark-adapted, while in other instances both eyes became light-adapted. Bennitt concluded that pigment movements in the two eyes of a crustacean were interrelated and that stimulation of one eye would bring about pigment migrations in both eyes; he suggested that the two eyes might be interrelated through hormonal control.

The retinal pigments of many species of crustaceans exhibit a marked diurnal rhythm of migration; in some species a diurnal rhythm persists in constant light, and in others it continues in constant darkness. The three retinal pigments behave differently in the various species. The evidence has been summarized by Brown (1944) and by Kleinholz (1949*a*). The existence of these diurnal rhythms reveals that factors other than direct illumination cause the retinal pigments to migrate. In 1930 Welsh showed that when a dark-adapted Palaemonetes was placed in the light for twenty minutes and then returned to darkness, the migrations of the retinal pigment, which began under illumination, continued for the next twenty minutes in darkness, although the stimulus for migration was no longer present. These observations led Kleinholz (1936) to investigate a possible hormonal control of distal pigment movements in Palaemonetes. His injections of eye-stalk extracts into dark-adapted animals brought about the light-adapted position of the distal and the reflecting pigments, the proximal pigment remaining unaffected by the injected extract. Welsh (1939) has subsequently shown that injections of concentrated eye-stalk extracts will cause the proximal pigment of dark-adapted Cambarus to move into the position characteristically found in light-adapted animals. It has not yet been shown that in any one species the distal, reflecting and proximal pigments can all be moved by injections of hormones, and in some species one or another of these pigments remains in a fixed position, and is unaffected by injections or by change of illumination. It seems clear, however, that the three pigments of the crustacean eye will respond to injections of eye-stalk extracts, although these injection experiments alone do not determine that the control of the pigment movements is predominantly hormonal under normal conditions. The experiments described above, which indicated a partial independence of the covered and the exposed eye of an individual, showed that other factors besides hormonal ones were involved. In the light of so much confusing evidence it seemed desirable to investigate the matter, using *Leander serratus* as a test animal.

Part of the present work was done at Plymouth and the remainder at Naples. I should like to acknowledge my gratitude to the Challenger Society which defrayed many of my expenses at Naples, and to Professor A. C. Hardy, F. R. S., who kindly allowed me to use the Oxford table there. On one occasion I occupied the table at Plymouth rented by the British Association and on another occasion that rented by the Clothworker Company. I am greatly indebted to these two bodies for the use of their tables at Plymouth. I am also indebted to the Directors and staffs of the Laboratories of the Marine Biological Association at Plymouth, and the Stazione Zoologica at Naples for their unfailing courtesy and help.

Material and Methods

In the present experiments large male individuals of *Leander serratus* were used. In order to have some means whereby the positions of the distal retinal pigment

might be expressed numerically and compared in eyes of different size, the following procedure was adopted. Measurements were taken of the distance from the basement membrane to the distal ends of the distal retinal pigment cells, and also from the basement membrane to the surface of the eye (see text Fig. 1). The position of the distal pigment can thus be expressed numerically as the ratio of the former measurement to the latter, and is denoted in the present paper as the distal retinal pigment index; this measurement provides a satisfactory method of comparing the response of the distal retinal pigment in eyes of different size.

In those experiments which required an opaque cover for the retina some difficulty was experienced in finding a satisfactory substance; one which would not dissolve in sea-water and yet did not contain a solvent that would affect the retina. In a preliminary series of experiments the mixture of Canada Balsam and lampblack used by Parker (1897) was employed, but it was found that the xylene solvent itself caused positional changes in the retinal pigments and the use of this mixture was discontinued. Eventually red modelling clay (marketed under the name of Iwana) was found to be satisfactory for covering the eyes. In applying this clay it was found convenient to secure an animal by fixing two tapes over the body, one over the cephalothorax and the other over the abdomen, and pinning these to a small board. A bit of modelling clay was fashioned into a ring which was slipped over one eye and attached firmly around the exoskeletal base of the eye-stalk; a second ring was then slipped over the eye-stalk and by using the fine point of a needle was joined to the first ring; finally, a small cap of clay was fitted over the retinal portion of the eye and moulded to the second ring around the eye-stalk. In this way a loose opaque sac of modelling clay was fitted over the eye and its stalk. The smaller diameter of the eye-stalk prevented the cover from falling off.

An alternative method of covering one eye is the box method described by Parker (1897). The animal is fastened by tapes to the inside of a light-proof box in such a way that one eye-stalk projects through a hole just wide enough to admit it. The box is then placed under water in a darkroom with a beam of light directed on the exposed eye along a plane parallel to the side of the box; this direction is calculated to allow the least possible amount of light into the interior of the box. The box experiment and the method of covering the eye with clay provide two contrasts in method. In the box method the covered eye is not constrained in any way, whereas in the other experiment it bears a clay covering. In the clay experiment the body is illuminated but in the box experiment it is not.

The intensity of light in these experiments was measured by a Weston photometer, which expressed light-intensity in terms of seconds necessary for photographic exposure. This did not give an accurate absolute measurement but was sufficiently sensitive to permit an adjustment of the source of illumination so that approximately the same intensity of light fell on the eye in each separate experiment. The temperature of the water was taken before and after each experiment. In no case had the temperature risen more than 2.0° C. at the end of a four-hour interval. Black, white and grey backgrounds consisting of painted cake tins were used in some of the experiments.

Removal of the sinus-glands in some of the experiments was accomplished by a modification of the method described by Panouse (1946). The animal to be operated upon was secured by tapes as described above, a piece of modelling wax was arranged at one side of the head and a groove was made in the wax in which

one eye could rest. The operations were generally performed in the evening after the animals had been in darkness for some hours so that the contraction of the chromatophores on the eye-stalk allowed the sinus-gland to be seen clearly through the cuticle of the stalk. The cuticle was lightly pierced above the gland with the points of fine watchmaker's forceps, and a small piece of the cuticle was removed; the aperture made was sufficiently large to allow the points of the forceps to pass through and seize the sinus-gland which is a bluish-white mass in contrast to the more translucent surrounding tissues (Fig. 2). After removal of the sinus-gland the hole in the cuticle was filled by a small plug of modelling clay which prevented any further loss of blood. In each animal excision of the second sinus-gland was performed 24 hours after removal of the first gland.

The success of sinus-gland removal was judged by (1) direct observation of the eye-stalk, (2) the behaviour of the dark pigments in the chromatophores, (3) sectioning the eye-stalks after experiment. Immobility of the dark chromatophoral pigments for ten days, under various conditions of illumination and background, was taken as an indication of successful sinus-gland removal. Later sectioning of the eye-stalks confirmed this opinion. About one-third of the operations were successful, a figure which is in agreement with those obtained by Panouse (1946) and Brown, Ederstrom and Scudamore (1939). The mortality among operated animals was about 25 per cent. In all, 148 experimental and 18 control animals were examined.

A preliminary series of experiments revealed a diurnal rhythm of activity of the proximal and reflecting pigments (Fig. 3). In constant darkness, these pigments became more completely dark-adapted at midnight than they did at midday. To avoid the extremes of this rhythm, all subsequent experiments were carried out during the afternoon and in no cases finished later than 6 p.m.

After each experiment the animals were immersed in hot water (80° C.) for 10 seconds to fix the position of the retinal pigments. The eyes were excised and transferred to a modified Bouin's solution (containing 7 per cent acetic acid) for 24 hours. They were then placed successively in 70 per cent alcohol for 3 hours, in dioxane for 12 hours and in paraffin wax for 3–5 hours. Before the final embedding, the eyes were oriented in the melted paraffin with the dorsal side uppermost (readily recognized by the black pigment-spot located at the junction of the retinal and non-retinal portions of the eye-stalk). Serial sections were cut at 15–20 μ and were generally mounted unstained for observation of the position of the retinal pigments. Measurements of the position of the distal pigment were made on the central ommatidia of those sections bearing the dorsal pigment spot; thus the measurements represent, as nearly as possible, the position of the distal pigment cells in the central ommatidia of the eye.

The Eye of Leander Serratus

The structure of the decapod eye has been described in detail by many authors; a survey of the literature on the subject has been given by Parker (1932) and a more recent account of the structure of the eyes of some species has been given by Debaisieux (1944). There is, however, a lack of uniformity in the terminology used by different authors to describe corresponding parts of the eye in different species.

The eye of *Leander serratus* is composed of about 7000 ommatidia and a dorsal accessory pigment spot. Each ommatidium (Fig. 1) comprises five cell-types as

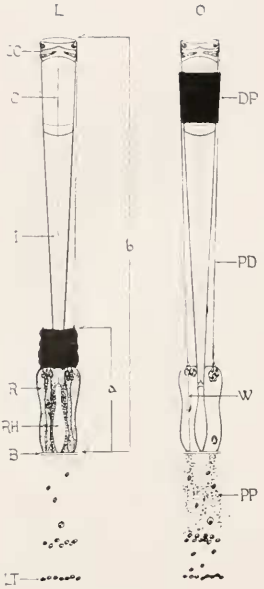

FIGURE 1.

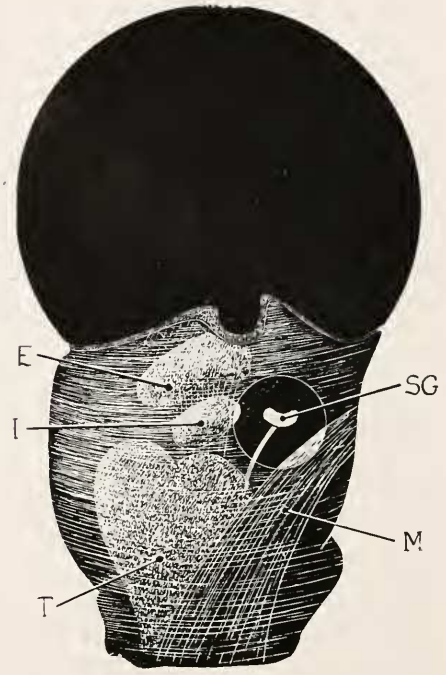

FIGURE 2.

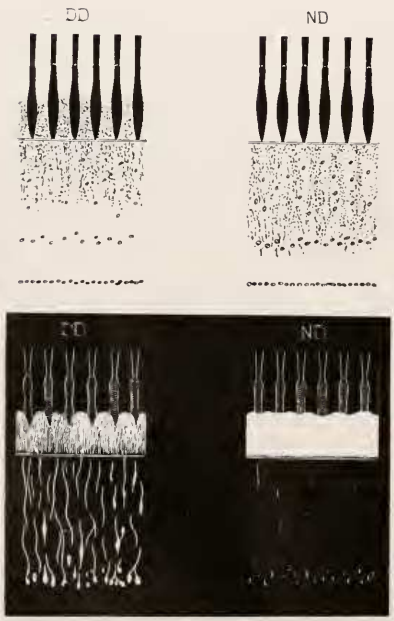

FIGURE 3.

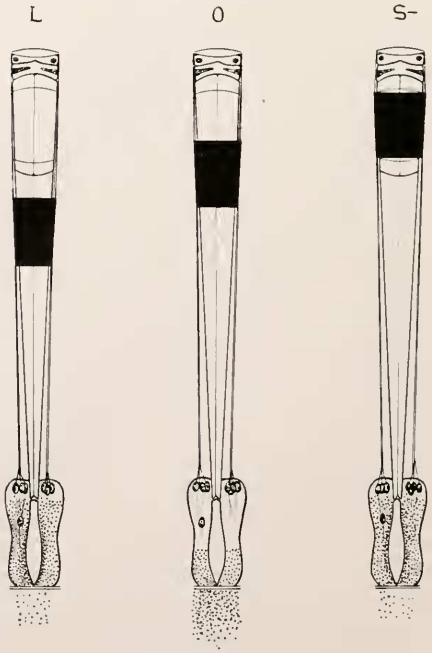

FIGURE 4.

follows: (a) Corneal hypodermal cells which secrete the cornea. (b) Cone cells which form a crystalline cone. (c) Distal retinal pigment cells. (d) Proximal pigmented retinular cells, which surround a central transparent mass, the rhabdome. (e) Tapetal pigment cells, of which there is one cell to a number of ommatidia.

The cornea is facetted in such a way that each ommatidium bears, at its distal end, one facet which is square in surface view and slightly biconvex when viewed in section. Each facet is composed of a thin scaly epicuticle and a thicker cuticle. Two corneal hypodermal cells lie beneath each facet.

The cone consists of four elements which form an elongated pyramid with its square base approximated to the proximal surfaces of the corneal hypodermal cells. In this basal portion there are four nuclei, corresponding to the four elements of the cone. Each cone element is not homogeneous throughout its length, but comprises a cell-body, the crystalline cone proper, and an extension which Debaisieux (1944) calls an intermediate crystalline tract. Distally, prolongations of the cone cell-bodies appear to pass between the corneal hypodermal cells and may come in contact with the corneal facet. Proximally the intermediate crystalline tract is prolonged into a number (Debaisieux states that there are 11 to the four crystalline tracts in *Palaemon varians*) of fine fibres which extend proximally between the retinular cells and envelop the rhabdome; in some species these fibres extend to the basement membrane, but in Leander neither Schneider (1902) nor Trojan (1913) could trace them as far as this.

The cone is surrounded by two pigment cells which have been known variously as "Hauptpigmentzellen," "Iris pigment cells," "Distal retinular cells," and "Pigmentzellen." The name "Distal pigment cell" has been widely adopted in recent literature and will be used in the present account. These distal cells contain mainly dark pigment granules, but in addition there is a thin covering sheath of white light-reflecting pigment. The distal and the proximal extensions of the distal pigment

FIGURES 1–4.

FIGURE 1. Light adaptation in the eye of *Leander serratus*. Two ommatidia are shown, the left in a condition of total light-adaptation, the right with the pigments in a dark-adapted position. The change in position of the distal and proximal pigments is depicted; the position of the reflecting pigment is not shown. The measurement a/b is described in the text as the distal pigment index.

CC. Cells of crystalline cone. C. Cone. I. Intermediate crystalline tract. R. Retinular cell. RH. Rhabdom. B. Basement membrane. LT. Lamina ganglionaris. DP. Distal pigment. PD. Proximal extension of distal pigment cell. W. Distal extension of reflecting pigment cell. PP. Proximal pigment.

FIGURE 2. The position of the sinus-gland in the eye-stalk of *Leander serratus*. The right eye is viewed from above, and a small aperture has been cut in the cuticle directly above the sinus-gland. M. Muscle. SG. Sinus-gland. E. Medulla externa. I. Medulla interna. T. Medulla terminalis.

FIGURE 3. Diurnal rhythm of proximal and reflecting pigments in *Leander serratus*. The movements of the proximal pigment are shown in the upper drawings; the reflecting pigment is depicted in the lower figures. On the left, the retina of an individual killed at midday after 24 hours in constant darkness (DD). On the right, the retina of an animal killed at midnight after 12 hours in constant darkness (ND).

FIGURE 4. Illumination and the sinus-gland hormone in the movements of the distal pigment in *Leander serratus*. L and O are ommatidia from the exposed left and covered right eye respectively of an animal maintained in a box with one eye projecting through a hole in the side of the box. S- is an ommatidium from an individual from which the sinus-gland had been removed a week previously, and which had been for four hours exposed to powerful illumination.

FIGURE 5.

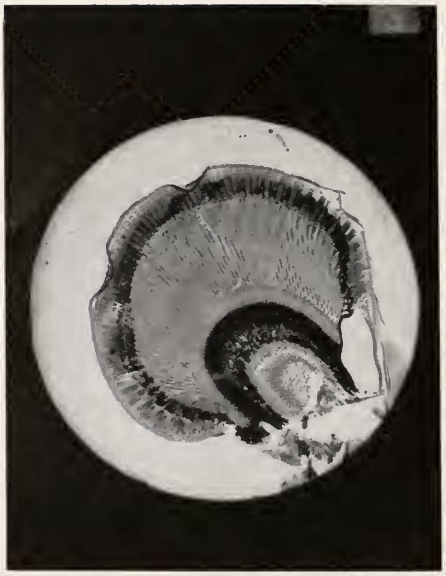

FIGURE 6.

FIGURE 7.

FIGURE 8.

FIGURE 9.

cells have been variously described in the literature. Welsh (1930) depicted a distal extension of the pigment cells to the cornea in *Palaemonetes vulgaris* and a proximal extension by fibres to the distal ends of the retinular cells. Debaisieux (1944) shows a distal extension to form a collar around the corneal hypodermal cells and proximal fibres extending to the basement membrane. In eyes containing both dark and white pigments the connections are not easily distinguished because the white pigment appears dark by transmitted light and, moreover, it is partly masked by the dark pigment. In my preparations of retinas of *Leander serratus* I have observed that if the dark pigment is removed by controlled immersion in dilute ammonia, but the white pigment remains, the extensions described by Debaisieux can be seen (Fig. 7). If the white pigment only is removed, by immersion in boiling water, the connections depicted by Welsh can be observed. I believe that the inner darkly pigmented portion of the distal cell is in continuity with the retinular cells proximally and the cornea distally, but the sheath of white pigment is in continuity with the white pigment of the tapetal layer proximally and the cornea hypodermal cells distally.

There are eight proximal retinular cells, though one of these is very reduced in size. Proximally these cells taper to nerve fibres which pass through the basement membrane and gather to form the lamina ganglionaris, whence fibres pass to the medulla externa of the eye-stalk. The extensions of the retinular cells between the basement membrane and the lamina ganglionaris are fairly wide, and pigment contained within the retinular cells migrates above or below the basement membrane in response to the illumination falling on the eye. The pigment contained in the retinular cells is known as the proximal pigment.

The tapetal layer comprises a number of nucleated cells, about one to each ten ommatidia, which lie distally to the basement membrane, but with processes extending proximally towards the lamina ganglionaris. The tapetal cells contain a white light-reflecting pigment, and have been compared by Debaisieux to the white chromatophores of the integument; distally they extend to the distal pigment cells and beyond. The pigment they contain is referred to as the reflecting pigment in this account.

Movements of the Retinal Pigments Under Normal Conditions of Illumination

To study first the normal migrations of the retinal pigments, on illuminated backgrounds and in darkness, the following procedure was adopted: Ten large male individuals of *Leander serratus* were placed in a dish on a white background and ten

Figures 5–9.

Figure 5. The exposed and illuminated right eye of an individual maintained in a light-proof box for three hours.

Figure 6. The covered left eye of the same individual shown in Figure 5.

Figure 7. A small portion of the retina of *Leander serratus* from which the dark proximal pigment has been removed. The white reflecting pigment appears dark by transmitted light. The distal extensions of the reflecting pigment cells are shown.

Figure 8. The retina of the exposed and illuminated eye of an individual kept in a light-proof box for three hours. The proximal pigment is in the extreme light-adapted position.

Figure 9. The retina of the darkened eye of the same individual as Figure 8. The proximal pigment is in the position of greatest dark-adaptation found in any animal killed during the day.

individuals were similarly placed on a black background; both sets of individuals were exposed to the same intensity of incident illumination. A third group of ten individuals was placed in darkness. The experiment was begun at 2 p.m. and continued until 6 p.m. when the animals were killed. Welsh (1930) reported that the average time required in *Palaemonetes vulgaris* for the distal pigment of a dark-adapted individual to become completely light adapted in the light was approximately 40 minutes, while about 90 minutes was required for the reverse process of dark-adaption. Migration of the proximal pigment is more rapid than that of the distal pigment. Although observers have given different times for the various species of decapods studied (Parker 1932), in no case do these estimates exceed 2 hours. The results of illumination and background experiments on the migration of the distal pigment are given in Table I.

TABLE I

The position of the distal retinal pigment under various conditions of illumination and background. Each experiment was carried out on ten animals. I, is the intensity of incident light measured in terms of seconds necessary for photographic exposure (see methods); R, is the intensity of reflected light. The standard deviation is calculated by the formula $S.D. = \sqrt{\frac{\Sigma D^2}{n}}$.

Light intensity			Nature of background	Average distal pigment index and standard deviation
I	R	$\frac{I}{R} \times 1000$		
.04	.33	120	white	.35±.067
.04	8	5	black	.41±.01
—	—	—	darkness	.75±.006

The distal pigment *indices*, for a second series of experiments carried out under similar conditions, ranged from .27–.3 for a white background, from .5–.6 for a black background and from .75–.90 in darkness. To test the value of this distal pigment index for comparing the position of the distal pigment in different species, the figures given by Kleinholz and Knowles (1938) were calculated in the same way. Treated thus, they gave a range of from .31 to .60 for eyes of animals placed on backgrounds ranging from white to black, and exposed to such conditions of illumination that the ratio, $\frac{I}{R} \times 1000$, ranged from 9 to 62. The figure for darkness was .76. It will be seen that these figures obtained in *Leander adspersus* correspond closely to those obtained in the present experiments on *Leander serratus*. It is thus evident that in these species the distal retinal pigment migrates distally in darkness and proximally under illumination and that the shade of the background influences the position of the distal pigment.

On the other hand, the proximal pigment in the retinular cells did not show differences in animals which had been on different backgrounds under illumination, though this pigment did, however, show differences between illuminated animals and those maintained in darkness. Under illumination the proximal pigment migrated distally so that it came to lie above the basement membrane, extending along the

rhabdom as far as the nuclei of the retinular cells; in darkness a great part of the proximal pigment migrated below the basement membrane and lay between it and the lamina ganglionaris.

Persisting Diurnal Rhythm

The retinal pigments of many crustaceans show a marked diurnal rhythm of migration, which may continue in constant light or in constant darkness in different species. The literature has been reviewed by Kleinholz (1942). Inasmuch as Panouse (1946) had reported that chromatophoral pigments of *Leander serratus* migrated diurnally in constant darkness, it seemed advisable to determine whether a persisting diurnal rhythm of the retinal pigments was present in this species. Fifteen individuals were taken from a tank and placed in darkness at 9 a.m. one day. At 12 noon on that day five of these individuals were killed, and the water changed for the others; at midnight the following night five more individuals were killed and the water once more replenished; at midday the following morning the remaining animals were killed. The eyes were compared after sectioning. No significant differences in the position of the distal pigment could be observed, but the positions of the reflecting and of the proximal pigments differed in animals which had been killed at midday or at midnight (Fig. 3). The reflecting pigment of animals killed at night had migrated distally to the basement membrane and lay between the retinular cells; on the other hand, in individuals kept in darkness and killed at midday, the reflecting pigment was dispered above and below the basement membrane. A similar condition was observed in illuminated animals killed at midday. The proximal pigment of Leander in darkness killed at night had migrated proximally to the basement membrane and lay between it and the lamina ganglionaris; while the proximal pigment of animals maintained in darkness and killed at midday lay partly below and partly above the basement membrane. It did not extend distally beyond the tapetal layer (about half the distance along the length of the rhabdome). In view of this persisting diurnal rhythm of proximal and reflecting pigment movements, all experiments were conducted at the same time each day.

The effect of covering one eye

In a preliminary series of experiments carried out using Parker's (1897) method of covering an eye with a mixture of Canada balsam and lampblack, animals thus treated were maintained for four hours on an illuminated white background. The striking results obtained by Parker were confirmed, i.e., the distal pigment of the covered eye moved into the dark-adapted position while that of the exposed eye remained light adapted. But subsequent experiments showed that Canada balsam alone had an effect on the distal pigment. A minute drop of Canada balsam, without lampblack, was applied to an eye and the animal thus treated was kept under observation. In less than half an hour the distal pigment cells of the ommatidia, directly beneath the Canada balsam, had migrated towards the periphery of the eye, indicating that the positional changes observed with this method were not due to opacity of the lampblack; probably the xylene penetrated the cornea and brought about the changes in position of the distal pigment.

(*a*) *Clay method.* A series of experiments was carried out using modelling clay as an opaque covering for the eye. The position of each pigment was recorded in each experiment and is given below and in Table II(a).

TABLE II

The effect of covering one eye and exposing the other to direct, incident and reflected illumination. In animals of the (a) series the eyes were covered with modelling clay after they had been kept on white backgrounds for three hours, and they were then placed on illuminated white and grey backgrounds. Animals of the (b) series were kept for three hours in darkness before their eyes were covered with modelling clay. Animals of series (c) were kept in a light-proof box as described in the text. The standard deviation has been calculated by the formula $S.D. = \sqrt{\frac{\Sigma D^2}{n}}$.

Numbers of animals	Conditions before experiment	Background	Time		Position of proximal pigment	Distal pigment index*
4	illumination	white	4 hours	exposed eye	light-adapted	.44±.048
(a)				covered eye	dark-adapted	.44±.069
6	illumination	grey	3 hours	exposed eye	light-adapted	.48±.060
				covered eye	dark-adapted	.51±.045
5	darkness	white	3 hours	exposed eye	light-adapted	.51±.027
(b)				covered eye	dark-adapted	.60±.031
5	darkness	black	3 hours	exposed eye	light-adapted	.59±.055
				covered eye	dark-adapted	.65±.060
3	illumination	—	2–3 hours	exposed eye	light-adapted	.49±.087
		—		covered eye	dark-adapted	.69±.098
(c)						
5	darkness	—	2–3 hours	exposed eye	light-adpated	.62±.071
		—		covered eye	dark-adapted	.69±.068

* An increase in the distal pigment index shows a movement towards the dark-adapted position.

(1) No changes were detected in the reflecting pigment.

(2) In all cases the proximal pigment of an exposed eye migrated distally in the retinular cells until it reached their distal extremities. On the other hand, the proximal pigment of the covered eye did not extend beyond the level of the tapetal layer, i.e., it occupied about half of the retinular cells above the basement membrane in each case. This is the position of the proximal pigment in animals maintained in darkness during the day. There was, therefore, a clear difference in the position of the proximal pigment in the exposed and the covered eye of each individual (Figs. 8 and 9).

(3) The positions of the distal pigments were observed under three different conditions of the animals. The first of these were animals which had been kept on illuminated white backgrounds before one of the eyes was covered, after which the animals were returned to illuminated white backgrounds. The distal pigments in these experiments showed no difference between the covered and the exposed eyes. In the second of these conditions the animals had previously been kept in darkness and one eye had been covered in very dim light. These animals were then kept on an

illuminated white background. In this case some slight difference between the covered and the exposed eyes was detected. Under the third condition the animals, previously kept in darkness, had one eye covered and were then placed on an illuminated black background. Here the difference in the position of the distal pigment in the exposed eye and the covered eye was significant. In many cases the distal retinal pigment index does not adequately represent this phenomenon, for the difference is a slight one, not easily expressed numerically, but clearly visible, especially in those animals in which the position of the distal pigment of both eyes approximated that found in darkness.

(*b*) *Box method.* The modelling clay method of covering the eye has two disadvantages: the body is exposed to illumination and light may enter the covered eye through the transparent eye-stalk. Verrier (1941) has reported that in *Leander squilla* covering of both eyes does not prevent light adaption of the distal retinal pigment if the body is exposed to illumination (her plates show that the proximal pigment of the covered eyes become partially dark-adapted like that of *Leander serratus* placed in darkness during the day). It is not surprising, therefore, that in the covered eye experiments described above the distal pigments generally became partially light-adapted.

In the box experiments, however, one eye only is exposed to illumination, while the rest of the body is in darkness. The results of these experiments are given in Table II(c). They differ from those in the previous experiments in that the distal pigment of the covered eye generally showed little or no light-adaption. The distal pigment of the illuminated eye showed a slight proximal migration which was always greater than that observed in the covered eye. (See Figs. 4, 5, and 6.)

The effect of sinus-gland removal

The effect of sinus-gland removal was studied in *Leander serratus* at Plymouth and in *Leander adspersus* at Naples. In some cases both sinus-glands were removed from an individual; in other cases the sinus-gland was removed from one eye-stalk and the other eye-stalk was ablated. The results obtained were substantially the same whichever method of sinus-gland removal was adopted. The animals were kept for ten days after the operation and then placed on a white background for 3–4 hours under powerful illumination. At the end of the experiment the animals were killed and the eyes were sectioned. A few animals without sinus-glands were kept in darkness as controls.

Migration of the proximal pigment was unaffected by the removal of the sinus-gland and invariably became light-adapted under illumination and dark-adapted in darkness. The extent of migration of this pigment was similar to that observed in normal individuals kept under similar conditions of illumination.

The position of the distal pigment in the eyes of the operated animals showed some variation in different individuals. In some individuals it attained the position of complete dark-adaptation (Fig. 4), even though the animals had been exposed to direct and powerful illumination; in some other individuals it underwent a slight proximal migration.

A few individuals of *Leander adspersus* were operated on for the removal of the sinus glands, and later, after a few days, one eye was covered with modelling clay. The results obtained by this means were similar to those obtained in *Leander serratus*

by the box method, although the migration of the distal pigment in the illuminated eye was greater than that observed in *Leander serratus*.

Discussion

The experiments which have been described in the present paper leave little room for doubt that the presence or absence of illumination has a direct effect on the cells containing pigment in the retina of *Leander serratus*. The migrations of the proximal pigment, and to a lesser degree those of the distal pigment, are greater in an exposed eye than in a covered one. It is necessary, therefore to re-examine the evidence for a humoral control of retinal pigment migration.

Evidence for the humoral control of proximal pigment migration depends on the injection experiments of Welsh (1939). An injection of eye-stalk extract caused the proximal pigment of Cambarus to migrate in darkness. It is clear from the experiments described above that the presence of the sinus-gland is not necessary for a migration of the proximal pigment in *Leander serratus* and *Leander adspersus*. Kleinholz (1948) has observed a similar persistance of proximal pigment migration after sinus-gland removal in Cambarus, and he has subsequently shown (1949b) that migrations of the proximal pigment, in response to illumination, continue in the isolated eye of Astacus in which the circulatory system no longer functions and the influence of the brain can no longer operate. The results obtained in the present experiments, by covering one eye, show that the proximal pigment responds directly to the presence or absence of illumination.

The evidence obtained from the study of persisting diurnal rhythms in *Leander serratus*, and in other species, shows that migration of the proximal pigment can take place in the absence of illumination. It is clear, therefore, that the control of the proximal pigment is a complex one, in which direct illumination is not the only factor.

The effect of injection experiments and sinus-gland removal show that the control of the migration of the distal retinal pigment is predominantly humoral. It is clear also, from the immobility of the distal pigment in certain individuals following sinus-gland removal, that the distal pigment cannot respond directly to illumination by migrating proximally. There remains, therefore, the puzzling dissimilarity between the exposed and covered eyes of single individuals in respect to their distal pigments (Fig. 4.). The present evidence suggests that light does have a direct effect on the distal pigment, but *only when the sinus-gland hormone is circulating in the blood stream*. If this is indeed the case, it is possible that light is in some way sensitizing the distal pigment cells to the hormone by which they are activated. Such a variation in the response of an effector to its activator is unusual and merits further investigation.

Summary

1. The distal and the proximal retinal pigments of *Leander serratus* can alter their positions in response to light and darkness.

2. The reflecting pigment shows a great movement at night which persists as a diurnal rhythm in continued darkness, but during the day it is unaffected by change of illumination.

3. The extent of distal pigment migration depends on the shade of the background on which the animal is placed, if the intensity of incident illumination is constant.

4. In constant darkness the proximal pigment continues to migrate diurnally for some days, at least. At midnight it shows a greater degree of dark-adaption than it does during the day; the distal pigment cells do not exhibit any detectable persisting diurnal rhythm in continued darkness.

5. After removal of the sinus-gland the distal pigment, in most of the operated animals, attained the position of maximal dark-adaption and was unaffected by change of illumination; it therefore appears not to respond directly to illumination. On the other hand, the proximal pigment in animals without sinus glands exhibited the same responses to change of illumination as those observed in normal animals.

6. In a series of experiments one eye of an animal was covered and the other eye was illuminated. In all the experimental animals so treated the proximal pigment of the exposed eye became light-adapted, but that of the covered eye attained the position of dark-adaptation observed in animals kept in darkness during the day.

7. A slight difference between the position of the distal pigment in the exposed eye and the covered eye of an individual could sometimes be observed; the difference was most striking in those animals which were on a black background, and in those animals which were placed in a light-proof box (in darkness) with one eye protruding through the box to be illuminated.

8. The distal retinal pigment of *Leander serratus* does not respond directly to illumination when the sinus gland is absent, yet in normal animals direct illumination enhances the light adaptation of the distal pigment; possibly illumination sensitizes the distal pigment cells to the hormone by which they are activated. The migration of the proximal pigment under illumination appears to be independent of the sinus gland. The influence of direct illumination on the proximal pigments appears to be great.

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